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BRCA MUTATIONS: REPRODUCTIVE POTENTIAL OF CARRIERS

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Contents

ABSTRACT

BRCA1 and *BRCA2* genes are tumour suppressor genes encoding proteins vital to the maintenance of genomic integrity. Germline mutations in these genes are the most relevant known causes of inherited susceptibility to breast and ovarian cancers, and also, to a minor degree, other forms of cancer. The disclosure of a *BRCA* pathological mutation leads to a difficult decision-making progress on the patient's behalf as they are given multiple options regarding both screening and therapeutic approach. Considering the possible life-changing and permanent results from these decisions, the counselling of *BRCA* carriers must integrate the most current knowledge in its practice. When facing this process, one main concern of these women is their individual family formation plan, thus the importance of providing specific information having in account their *BRCA* status.

This review aims to contribute to clarifying the possible association between *BRCA1*/*BRCA2* pathological variant carrier status and the modification in the reproductive potential through the evaluation of the impact of such mutations on ovarian reserve, gravidity and parity, and menopause onset age.

A literature search was conducted on the databases PUBMED, Up-To-Date and Cochrane Library, using combinations of the Mesh Terms and keywords “fertility” and “*BRCA1* gene” or “*BRCA2* gene” or “*BRCA*”, selecting relevant articles published over the last decade.

The available clinical data on this matter has presented conflicting results, although there is a trend supporting the consideration of fertility impairment as an additional consequence and outcome of *BRCA* mutations, bringing up the need to include fertility preservation strategies in the management of these patients.

KEYWORDS

FERTILITY; *BRCA1*; *BRCA2*; *BRCA*

Os genes *BRCA1* e *BRCA2* são genes supressores tumorais que codificam proteínas vitais para a manutenção da integridade genómica. Mutações germinativas nestes genes são a mais relevante causa conhecida de susceptibilidade hereditária para cancro da mama e ovário, e também, em menor grau, outras formas de cancro. A revelação de uma mutação *BRCA* patológica leva a um difícil progresso de decisão por parte dos doentes pois são oferecidas múltiplas opções tanto de vigilância como de terapêuticas. Tendo em conta os possíveis resultados permanentes dessas decisões com impacto drástico na vida da doente, o aconselhamento de portadoras *BRCA* deve integrar o conhecimento mais recente na sua prática. Quando confrontadas com este processo, uma das principais preocupações destas mulheres é o seu plano individual de formação familiar, pelo que é importante providenciar informação específica tendo em conta o seu estado *BRCA*.

Esta revisão visa contribuir para a clarificação da possível associação entre ser portador de variante *BRCA1/BRCA2* patológica e a alteração do potencial reprodutivo através da avaliação do impacto de tais mutações na reserva ovárica, gravidez e paridade, e idade de manifestação da menopausa.

Uma pesquisa bibliográfica foi realizada nas bases de dados PUBMED, Up-To-Date e Cochrane Library, recorrendo a combinações dos termos Mesh e palavras chave “fertilidade” e “gene BRCA1” ou “gene BRCA2” ou BRCA, selecionando artigos relevantes publicados na última década.

A informação clínica disponível sobre esta matéria apresenta resultados discordantes, apesar de se verificar uma tendência para o apoio da consideração de diminuição da fertilidade como uma consequência adicional e resultado de mutações *BRCA*, criando a necessidade de inclusão de estratégias de preservação de fertilidade na gestão destes doentes.

PALAVRAS-CHAVE

FERTILIDADE; BRCA1; BRCA2; BRCA

INTRODUCTION

BRCA

Scientific knowledge is in constant evolution, having been achieved major developments on various fields over the last few decades. Medicine has taken advantage from this, allowing the attainment of new data and the development of new techniques. Along with the improvement of other domains, this has allowed for the increase of human life expectancy at birth[1], raising new issues regarding the provision of health care as demographic changes take place.

The progress in genetics and the development of the branch of medical genetics have allowed a better understanding of the most diverse pathologies and a more individualized management of the patients affected by them, with special impact in diagnosis, treatment and prognosis, as well as prevention, allowing for counselling and taking action before the pathologic condition has settled.[2]

The field of oncology has specially benefited from the development of genetics, with the discovery of germline mutations in inherited cancer predisposition genes from the late 1980's to the present. Most of these are characterized by high penetrance, meaning that there is a high correlation between the presence of the genetic mutation and the development of disease, and relatively low incidence in the population, accounting for approximately 5-10% of all cancers.[3]

Germline mutations in the *BRCA* genes, *BRCA1* and *BRCA2*, are the most important known causes of inherited susceptibility to breast and ovarian cancers, also referred to as hereditary breast-ovarian cancer (HBOC), being also associated with, albeit to a minor degree, other forms of cancer, such as prostate, pancreatic, melanoma and Fanconi anemia.[47,48,49,50] The hereditary of pathogenic variants of these genes is attributable to a dominant autosomal pattern of inheritance.

BRCA1 and *BRCA2* genes, located on chromosomes 17q21 and 13q12 respectively, are tumour suppressor genes encoding proteins vital to the maintenance of genomic integrity by intervening in the process of DNA repair, through repair of DNA double-strand breaks, and in the transcriptional and cell cycle regulation[4].

The prevalence of pathogenic variants in these genes varies among populations, being estimated at 1:300 to 1:500 in the general population[5], reaching higher values among particular ethnic groups or individuals that currently are or were in the past geographically or culturally isolated, on account of the founder effect, in which at least

one of the ancestors was a carrier of the mutation. Several of these founder mutations have been observed worldwide, with the highest prevalence of 1:40 described amongst the Ashkenazim Jews.[6] Regarding the Portuguese population, three mutations with founder effects have been recently described in HBOC, two in *BRCA1* (c.2037delinsCC and c.3331_3334del) and one in *BRCA2* (c.156_157insAlu) accounting for about 50% of pathogenic mutations found in Portuguese families with HBOC.[7] The c.2037delinsCC *BRCA1* and c.156_157insAlu *BRCA2* rearrangements represent founder mutations specific to the Portuguese community.[7]

In clinical practice, the exact risk of developing cancer associated to the *BRCA* pathogenic variants is difficult to estimate as this depends on a multitude of factors in the context they are presented, such as the implicated genetic phenomena, age and sex of the patient, and medical and familiar history. Despite this limitation, an estimate of the malignancy risk can be provided according to the germline pathogenic variant gene of the individual. Compared to the 12% risk of developing breast cancer among general population, *BRCA1* and *BRCA2* mutation carriers are at a much higher risk, of around 46%-87% and 38%-84%, respectively, meaning a risk increase of about 3-7 times. Similarly, while the risk of development of ovarian cancer, including fallopian tube and primary peritoneal cancers, in the general population is of 1-2%, carriers of deleterious mutations in *BRCA1* or *BRCA2* face a risk of around 39-63% and 16,5-27%, respectively, of developing this type of cancer, an increase of maximum risk that can be roughly estimated up to around 63 times higher in *BRCA1* variants and up to around 27 times higher in *BRCA2* variants, when compared with general population.[8]

GENETIC COUNSELLING AND MANAGEMENT OF *BRCA* MUTATIONS

The knowledge about *BRCA* deleterious mutations gathered in the last decades has served as background to the development of more suitable guidelines and clinical management strategies for patients at risk of HBOC, being currently emphasised the importance of molecular genetic testing of *BRCA1* and *BRCA2* gene panels in patients with personal or family history suggestive of the presence of variants in these genes, such as the diagnosis of breast cancer before the age of 50, the diagnosis of ovarian cancer, the existence of two or more direct relatives with breast cancer, having at least one of the diagnosis been made under the age of 50, among other criteria consensually used by medical societies and associations worldwide.[8,9,10]

Molecular genetic testing in the context of patients diagnosed with breast and/or ovarian cancer is of great importance regarding the diagnosis, prognosis and therapeutic response. Moreover, the disclosure of a *BRCA* pathogenic mutation among women with cancer is of significance regarding therapeutic approach. Although most of the standard measures apply, recent findings suggest that BRCA positive breast and ovarian tumours may benefit from the use of PARP inhibitors.[51,52] Surgical methods are mostly regarded as first approach, with other therapeutics such as chemotherapy, radiotherapy and pharmaceutical treatment mainly regarded as coadjuvants. In patients with ovarian cancer, the recommended surgical approach consists of total hysterectomy and bilateral adnexectomy[9,10], resulting in loss of the reproductive capacity; whilst other surgical options exist, such as tumour reduction or conservative approach, regarded as a second option for women with the desire to preserve the fertility, these are not advised in women with HBOC history.[9] In pre-menopausal patients with either breast or ovarian cancer, chemotherapy is associated with possible side effects such as premature ovarian failure, with consequent loss of fertility. Although recent research has shown promising results in order to prevent such side effect[11], it should not be disregarded. In fact, fertility preservation by oocyte preservation is nowadays an option for those reproductive aged women.[54]

The identification of a *BRCA* deleterious variant in women without a personal history of cancer compels the need for counselling as these women face an arduous decision making process regarding the risk management options, in terms of both prevention of primary manifestations and surveillance.

Regarding modifiable lifestyle choices, the data on the *BRCA* mutation carriers population are limited and care for further investigation. Current information suggests some possible associations which should be taken in account during individual counselling (Table 1).

MANAGEMENT OF OVARIAN CANCER RISK

Recommendations for the management of ovarian cancer risk focus on prophylactic surgery, advising bilateral adnexectomy (also addressed as bilateral salpingo-oophorectomy) once childbearing is complete, in the age range of 35 to 40 years old, having in mind the possible need for individualized range definition based on age of onset of ovarian cancer in the family. In *BRCA2* carriers, the average later onset allows for a delay in the bilateral adnexectomy until the age of 40 to 45. [8, 9, 10]

Table 1 – Factors with possible association with cancer risk[12]

		BRCA1	*BRCA2*
Breast cancer	Risk increase	• Oral contraceptives	• Smoking
	Risk decrease	• Age at first birth • Breastfeeding • Late age at menarche	
Ovarian cancer	Risk decrease	• Breastfeeding • Tubal ligation • Oral contraceptives	• Oral contraceptives

Ovarian cancer screening, although deemed not effective in detecting early stage cancer, may be considered and offered to women who, after *BRCA* deleterious variant disclosure, have elected not to undergo or to delay surgical risk reduction. This surveillance consists of transvaginal ultrasound and serum CA-125 concentration at clinician's discretion (annual frequency recommended), beginning at 30 to 35 years old or 5 to 10 years before the earliest age of onset of ovarian cancer in the family (table 2).[8,10]

Table 2 – Strategies of ovarian cancer risk management[8,10]

Management strategy	**Description**
Prophylactic bilateral adnexectomy	• Recommended in the age of 35 to 40 y.o. (possible individualized range definition based on age of onset of ovarian cancer in the family). • BRCA2 carriers – reasonable to delay until the age of 40 to 45
Ovarian cancer screening	• Beginning at 30 to 35 y.o. (possible individualization range definition – 5 to 10 years before earliest onset in the family) • Transvaginal ultrasound and serum CA-125 concentration at clinician's discretion (annual frequency recommended)

MANAGEMENT OF BREAST CANCER RISK

Concerning breast cancer risk, management recommendations include intensive screening in addition to consideration of hormonal and surgical forms of risk reduction (table 3).[8,10]

Breast cancer screening involves monthly self-breast exams, periodic medical appointments starting at age 25 allowing for clinical breast examination every 6 to 12 months and annual breast MRI, in addition to annual mammogram beginning at age 30. The age for beginning of screening can be individualized based on the earliest age of onset in the family.[8,10]

Prophylactic bilateral mastectomy is to be considered, being associated with a decrease of 90% in incidence of breast cancer.[8,10] In addition to this surgical approach, prophylactic bilateral adnexectomy before the age of 50 might also be discussed, although there is conflicting data on the value of this measure in reducing the risk of breast cancer.[8]

Chemoprevention with tamoxifen is also a possibility for women who have elected not to undergo prophylactic surgery, though there is limited data available supporting the existence of preventive benefit in *BRCA* mutation carriers, with suggested differential effect in favour of women with *BRCA2* deleterious variants.[8,10]

Table 3 – Strategies of breast cancer risk management[8,10]

Management strategy	Description
Breast awareness	• Beginning at 18 y.o. • Periodic and consistent self-breast exam
Clinical breast exam	• Beginning at 25 y.o. • Periodic medical appointments (every 6-12 months)
Screening	• Beginning at 25 y.o. (possible individualization – before earliest onset in the family) • Periodic breast MRI with contrast and/or mammography with consideration of tomosynthesis (every 12 months) • 25-29 y.o.: MRI (preferable) or mammogram • 30 -75 y.o.: MRI and mammogram • >75 y.o.: individualized management
Prophylactic mastectomy	• Discussion regarding degree of protection (90%), reconstruction options and risks
Chemoprevention (i.e. tamoxifen)	• Discussion of risks and benefits

The decision making process faced by *BRCA* carriers is complex considering the diverse options and factors to take in account. The risk reduction approaches are effective, thus their inclusion in management guidelines, nevertheless they are associated with morbidities.

Prophylactic mastectomy may affect libido, sexual functioning, and body image, impacting on the quality of life of these patients.[53]

Bilateral adnexectomy leads to premature menopause when performed in premenopausal women, thus resulting in increased risk for pathologies associated with menopause, such as osteoporosis and heart disease. An outcome that cannot be disregarded is the inherent loss of reproductive capacity, considering the fact that fulfilling family formation goals poses as a factor while management decision making.[13]

Chemoprevention with tamoxifen is associated with risk of thromboembolic events and endometrial cancer.[14] This approach is also associated with fertility impairment, posing another option that has an impact on family formation.

OVARIAN FUNCTION ASSESSMENT AND FERTILITY

Nowadays, infertility is a common condition with repercussions on multiple social levels, with rather demographic, psychological and medical implications. Defined by the World Health Organization as “failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (and there is no other reason, such as breastfeeding or postpartumamenorrhoea)”, the same organization estimates it to inflict over 10% of women worldwide.

Most cases of female infertility are caused by ovarian disorders, being classified by the WHO into 3 groups: hypothalamic pituitary failure, hypothalamic-pituitary-ovarian dysfunction and ovarian failure. This classification is useful for the diagnosis and definition of therapeutic approachs according to the underlying dysfunction.

There are various risk factors negatively associated with woman’s fertility, specifically with oocyte quality and quantity. These present major impact on the reproductive potential considering women are born with a limited oocyte pool that diminishes over lifetime until menopause, posing age as an important factor with negative association with fertility. This association carries an even higher impact after the mid-thirties.[15] Other known risk factors rely on direct cell damage, such as smoking, drug consumption, radiation, chemotherapy, and several pathologies with known effect on ovarian cells.[16,17]

The assessment of ovarian reserve constitutes a vital component of the infertility evaluation. Although there is no ideal assessment strategy, there are available a number of guidelines from organizations worldwide[17-19] which recommend the coordination of screening tests in order to obtain a highly reliable prediction of reproductive potential.

Considering the previously stated, age should be used as initial predictor of overall fertility. In order to predict low response rate to *in vitro* fertilization (IVF), recommended tests include Anti-Müllerian hormone (AMH) and follicle-stimulating hormone (FSH) levels, although these present limited value regarding prediction of outcome.[17-19]

AMH levels reflect the size of the primordial follicle pool, gradually declining with age and being undetectable at menopause.[20-22] This test, while useful in identifying reduced follicle pool in women with infertility, does not correlate with fertility potential in women without infertility issues.[23]

FSH levels reflect the follicle production of ovarian hormones, presenting a negative correlation between the two. Low levels of FSH in women without other endocrine pathologies indicate sufficient production of ovarian hormones in order to inhibit pituitary secretion of FSH; in women with reduced follicle and oocyte pool, the production of ovarian hormones is insufficient, resulting in a rise of FSH levels.[24]

OBJECTIVE

The present review aims to contribute to clarify the possible association between *BRCA1*/*BRCA2* deleterious mutation carrier status and the modification in the reproductive potential through the evaluation of the impact of such mutations on ovarian reserve, gravidity and parity, and menopause onset age.

Management of patients upon the disclosure of a *BRCA* pathological variant includes different options regarding therapeutic approach, most having as a common outcome the loss of reproductive potential at a younger age when compared with the general population. Considering that the individual reproductive plan may account for the decision-making process, *BRCA* carriers face the provision of group specific fertility information and counselling poses as essential in order to allow the patients to make educated decisions with lifelong consequences.

METHODS

A literature search was conducted on the data bases PUBMED, Up-To-Date and Cochrane Library, using combinations of the following Mesh Terms and keywords: "fertility" and "*BRCA1* gene" or "*BRCA2* gene" or "*BRCA*". The search was restricted to articles published within the last decade, starting on 2008, written in English or Portuguese, and limited to the human species. Relevant articles were identified by screening titles and abstracts and consequently by reading the full text. In addition, the references of the articles of interest were screened to identify additional relevant articles. The assessment of the articles was made through the application of the PICO process.

Table 4 – PICO process applied in the assessment of the articles of interest)

P	Female carriers of *BRCA1*/*BRCA2* deleterious variants
I	Non-subject to prior therapeutic associated with fertility impairment
C	Female non-carriers of *BRCA1*/*BRCA2* deleterious or without *BRCA* status disclosure
O	Reproductive potential
Investigation question	Do female carriers of *BRCA1*/*BRCA2* deleterious variants, non-subject to prior cancer treatment, present differential reproductive potential (compared to female non-carriers)?

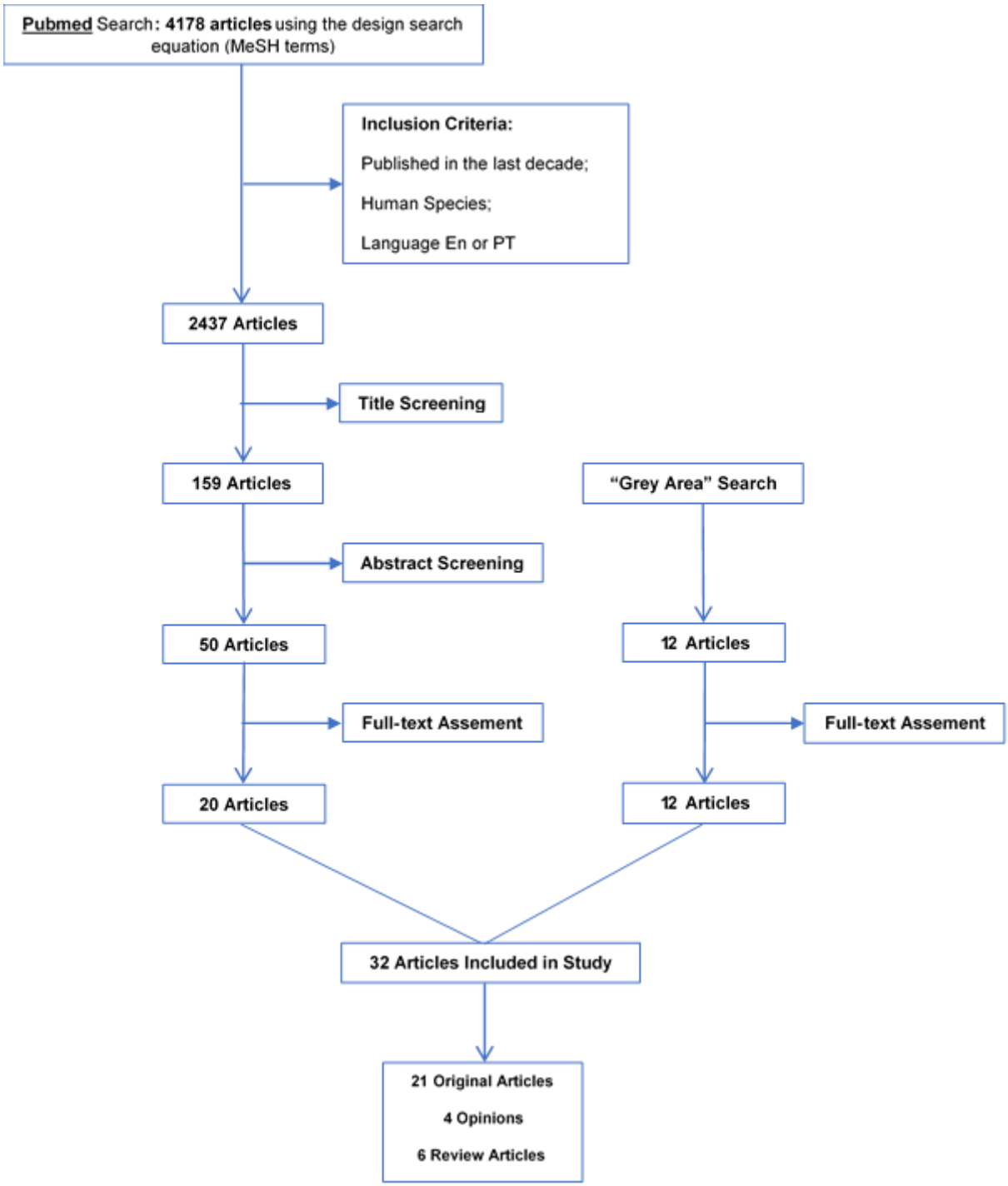

Figure 1 – Flow chart of article assessment

RESULTS

The Pubmed search yielded 4178 articles from which 24 were selected after the application of the eligibility criteria, consisting of 15 original articles, 5 reviews and 4 opinion articles; additionally, 3 original articles found in the Cochrane Library search and 9 articles obtained through other search means were also considered of interest for the present review.

The assessment of the articles of interest led to the definition of assorted key topics on which to focus based on the main and secondary outcomes in study. The full assessment of the articles, complete with study limitations description, can be found in tables 5-7.

Table 5 – Assessment of original articles of interest

AUTHOR	TITLE	OUTCOMES MEASURED					MAIN RESULTS AND LIMITATIONS
		OVARIAN RESERVE			GRAVIDITY AND PARITY	MENOPAUSE ONSET AGE	
		AMH LEVELS	FSH LEVELS	CRYOPRESERVATION STRATEGIES OUTCOMES			
Lauren J, et al Fertility and Sterility 2017	**ANTIMULLERIAN HORMONE LEVELS ARE LOWER IN *BRCA2* MUTATION CARRIERS**[28]	X			X		• **Negative association between *BRCA* positive status and history of infertility** • *BRCA1* carriers had higher percentage of history of infertility vs low-risk controls • *BRCA2* carriers had higher percentage of history of infertility vs low-risk controls • **No association between *BRCA1* status and AMH levels** • **Negative association between *BRCA2* status and AMH levels** • *BRCA2* carriers had lower median AMH levels vs low-risk control • *BRCA2* carriers had lower AMH levels throughout lifespan vs low-risk control • **Negative association between hormonal contraceptive use and AMH levels** • **Negative association between age (≥26y.o.) and AMH levels** • *BRCA2* carriers had a stronger association • **No association between BMI and AMH levels** • **No association between history of cancer and AMH levels** **Limitations:** cohort disparity (*BRCA* carriers statistically significantlyly younger vs low-risk control); possible bias for exclusion of *BRCA1* carriers with early onset disease and women >45y.o.

Giordano S., et al Journal of Adolescent and Young Adult Oncology 2016	**ASSOCIATION OF *BRCA1* MUTATIONS WITH IMPAIRED OVARIAN RESERVE: CONNECTION BETWEEN INFERTILITY AND BREAST/OVARIAN CANCER RISK[27]**	**X**			**X**		• **Negative association between *BRCA1* positive status and AMH levels** • *BRCA1* carriers had lower AMH levels vs non-carriers • **Negative association between age and AMH levels on *BRCA1* carriers** • *BRCA1* carriers >35y.o. had 10x the odds of low AMH vs *BRCA1* carriers <35y.o. (no association in non-carriers) • **Negative association between duration of oral hormonal contraception and AMH levels in *BRCA1* carriers (no association in non-carriers)** • 5 years on oral hormonal contraception increased 5x the odds of low AMH • **Weak association between parity and AMH levels in *BRCA1* carriers (no association in non-carriers)** • **Negative association between *BRCA1* positive status and parity** • *BRCA1* carriers more likely to be nulliparous vs non-carriers • *BRCA1* carriers less like to have had full-term pregnancy vs non-carriers **Limitations:** cohort disparity (*BRCA1* carriers younger vs non-carriers); different AMH assays (stored serum vs blood samples at first visit)
Wang E. T., et al Fertility and Sterility 2014	***BRCA1* GERMLINE MUTATIONS MAY BE ASSOCIATED WITH REDUCED OVARIAN RESERVE[26]**	**X**			**X**		• **Negative association between *BRCA1* positive status and AMH levels** • *BRCA1* carriers had 4,22x odds of lower AMH levels vs non-carriers • **No statistically relevant association between *BRCA2* positive status and AMH levels** • **No statistically relevant association between gravidity and**

							***BRCA* status** • Gravidity was similar in *BRCA1* carriers, *BRCA2* carriers and *BRCA* non-carriers • **Negative association between age and AMH levels** • **Negative association between BMI and AMH levels** **Limitations:** sample size (143 women, 62 *BRCA1* carriers vs 27 *BRCA2* carriers vs 54 non-carriers); high prevalence of Ashkenazi Jewish background; cohort disparity (*BRCA* carriers younger vs non-carriers); disregard of possible confound effect of hormonal contraception and smoking
Turan V., et al Reproductive Sciences 2017	**OVARIAN STIMULATION IN PATIENTS WITH CANCER: IMPACT OF LETROZOLE AND *BRCA* MUTATIONS ON FERTILITY PRESERVATION CYCLE OUTCOMES**[35]			**X**			• **Negative association between *BRCA* positive status and cryopreservation strategies outcomes** • *BRCA* carriers had lower number of oocytes retrieved and embryos frozen vs non-carriers • *BRCA* carriers had lower number of oocytes retrieved and embryos frozen vs untested women • Combined protocol of Letrozole + rFSH resulted in improvement of cryopreservation outcome in *BRCA* carriers vs protol of rFSH alone **Limitations:** unrandomized sample; cohort disparity (protocols applied to patients with different cancer types)
Finch A., et al Fertility and Sterility 2013	**FREQUENCY OF PREMATURE MENOPAUSE IN WOMEN WHO CARRY A *BRCA1* OR *BRCA2* MUTATION**[37]				**X**	**X**	• **Negative association between *BRCA* positive status and menopause onset age** • *BRCA* carriers had lower menopause onset age vs non-carriers • *BRCA1* carriers had lower menopause onset age vs non-carriers

							• *BRCA2* carriers had lower menopause onset age vs non-carriers • Higher percentage of *BRCA* carriers had menopause onset age <40y.o. • **No statistically relevant association between *BRCA* status and parity** • **No statistically relevant association between *BRCA* status and self-reported fertility** **Limitations:** data obtained through self-report questionnaire (recall may not be accurate), potential bias due to exclusion of women who may experience late menopause
Michaelson-Cohen R., et al International Journal of Gynecological Cancer 2014	**_BRCA_ MUTATION CARRIERS DO NOT HAVE COMPROMISED OVARIAN RESERVE[29]**	**X**					• **No statistically relevant association between *BRCA* status and AMH levels** • No difference in mean AMH levels in *BRCA* carriers vs untested women with • normal ovulatory cycles • Higher AMH in *BRCA1* carriers vs *BRCA2* carriers, without statistic relevance **Limitations:** sample size (364 women, 25 *BRCA1* carriers vs 12 *BRCA2* carriers vs 3 *BRCA1* and *BRCA2* carriers vs 324 untested women with normal ovulatory cycles); carriers sample limited to women with ≥1 Ashkenaki Jewish founder mutation
Collins I. M., et al. Journal Of Clinical Oncology	**DO *BRCA1* AND *BRCA2* MUTATION CARRIERS HAVE EARLIER NATURAL MENOPAUSE THAN THEIR NONCARRIER RELATIVES?**				**X**	**X**	• **No statistically significantly association between *BRCA* status and parity** • **No statistically significantly association between *BRCA* status and natural menopause onset age** • **Negative association between smoking and natural menopause onset age** (regardless of *BRCA* status)

2013	**RESULTS FROM THE KATHLEEN CUNINGHAM FOUNDATION CONSORTIUM FOR RESEARCH INTO FAMILIAL BREAST CANCER[38]**						**Limitations:** potential bias due to low percentage of the sample who reached natural menopause (19%)
Kim J., et al 2013	**BASELINE E2 LEVELS ARE HIGHER IN *BRCA2* MUTATION CARRIERS: A POTENTIAL TARGET FOR PREVENTION?[33]**		**X**		**X**		• **No statistically relevant association between *BRCA* status and FSH levels** • Basal FSH levels were not different in *BRCA* carriers vs non-carriers • **Positive association between *BRCA2* positive status and estradiol levels vs *BRCA1* carriers** • **Positive association between *BRCA2* positive status and estradiol levels vs non-carriers** • **No statistically relevant association between *BRCA* status parity** **Limitations:** sample size (96 women, 21 *BRCA1* carriers vs 8 *BRCA2* carriers vs 67 non-carriers), disregard of possible confound effect of breast cancer diagnosis on the hormonal variability
Tilborg T. C., et al Menopause: The Journal of The North American Menopause Society	**DO *BRCA1*/2 MUTATION CARRIERS HAVE AN EARLIER ONSET OF NATURAL MENOPAUSE?[41]**				**X**	**X**	• **No association between *BRCA* status and menopause onset age** • *BRCA* carriers did not have increased risk of earlier menopause vs non-carriers • *BRCA1* carriers did not have increased risk of earlier menopause vs *BRCA2* carriers • **Negative association between *BRCA* positive status and parity** • *BRCA* carriers were more often nulliparous vs non-carriers **Limitations:** data obtained through self-

2016							report questionnaire (recall may not be accurate), potential bias due to bilateral adnexoctomy uptake among *BRCA* carriers and over selection of breast cancer cases among non-carriers
Mancini J., et al Familial Cancer 2014	**IMPACT OF *BRCA1*/2 MUTATION ON YOUNG WOMEN'S 5-YEAR PARENTHOOD RATES: A PROSPECTIVE COMPARATIVE STUDY (GENEPSO-PS COHORT)[40]**				**X**		• **No statistically relevant association between *BRCA* mutation disclosure and gravidity/parity** • Motherhood rates were non-significantly lower among nulliparous *BRCA* carriers vs among nulliparous non-carriers • Motherhood rates were non-significantly lower among nulliparous *BRCA* carriers vs among *BRCA* carriers with ≥1 child at time of disclosure **Limitations:** sample size insufficient to reach statistical significance; disregard of possible confound effect of individual family formation goals or sexuality
Tilborg T. C., et al Human Reproduction 2016	**SERUM AMH LEVELS IN HEALTHY WOMEN FROM *BRCA1*/2 MUTATED FAMILIES: ARE THEY REDUCED?[30]**	**X**			**X**		• **No association between *BRCA* status and AMH levels** • Linear regression showed no reduction in AMH levels in *BRCA* carriers vs non-carriers • **No association between *BRCA* status and gravidity or parity** • Median age at first/last child and prevalence of miscarriage was not statistically significantly between *BRCA* carriers vs non-carriers • Statistically relevant higher self-report of infertility in *BRCA* carriers vs non-carriers **Limitations:** demographic disparity among cohorts (*BRCA* carriers younger vs non-carriers); funding/possible competing interests related to pharmaceutical industry
Pal T., et al	**FERTILITY IN WOMEN**				**X**		• **No association between *BRCA***

Fertility and Sterility 2010	**WITH *BRCA* MUTATIONS: A CASE-CONTROL STUDY[43]**						**status and parity** • Median age at first/last child and prevalence of miscarriage was not statistically significantly between *BRCA* carriers vs non-carriers • Statistically relevant higher self-report of infertility in *BRCA* carriers vs non-carriers **Limitations:** demographic disparity among cohorts (*BRCA* carriers younger vs non-carriers)
Moslehi R., et al American Journal Of Human Biology 2010	**IMPACT OF *BRCA* MUTATIONS ON FEMALE FERTILITY AND OFFSPRING SEX RATIO[39]**				**X**		• **No statistically significantlyly association between *BRCA* status and gravidity/parity** • The combined group of *BRCA* carriers and non-carriers had significantly relevant lower pregnancy rate vs controls **Limitations:** sample limited to Ashkenazi women; data based on self-report
Smith K. R., et al. Proc. R. Soc. B 2012	**EFFECTS OF *BRCA1* AND *BRCA2* MUTATIONS ON FEMALE FERTILITY[44]**				**X**		• **Positive association between *BRCA* positive status and parity** • Pre-1930 born carriers had 3,6x the odds to have ≥4 children vs controls • Post-1930 born carriers had 2,04x the odds to have ≥4 children vs controls **Limitations:** sample limited to married women born before 1974, on a limited geographic area (Utad or Idaho) with age of deah ≥45y.o.; *BRCA* mutation status obtained through genetic testing and ancestry analysis (putative); control group comprehends unlikely carriers (without genetic confirmation)
Kwiatkowski F., et al. PLOS|ONE	***BRCA* MUTATIONS INCREASE FERTILITY IN FAMILIES AT HEREDITARY**				**X**		• **Positive association between *BRCA* positive status and parity** • Rate of miscarriages was lower in *BRCA* carriers vs non-carriers • Average number of children

2015	**BREAST/OVARIAN CANCER RISK[45]**						per potential mother was higher in *BRCA* carriers vs non-carriers with *BRCA* family history; no statistical significance between *BRCA* carriers vs non-carriers without *BRCA* family history • Lower percentage of nulliparous among *BRCA* carriers **Limitations:** sample limited to French families with >5 members; disregard of possible confound effect of contraception access and individual family formation goals
Grynberg M., et al. Oxford University Press 2018	***BRCA1*/2 GENE MUTATIONS DO NOT AFFECT THE CAPACITY OF OOCYTES FROM BREAST CANCER CANDIDATES FOR FERTILITY PRESERVATION TO MATURE IN VITRO[32]**	**X**		**X**			• **No statistically relevant association between *BRCA* status and AMH levels** • **No association between *BRCA* status and number of *in vitro* matured oocyte preserved** • *BRCA* carriers had similar number of *in vitro* matured oocyte vs non-carriers **Limitations:** disregard for cancer status as a confounder; possible bias due to exclusion of women with ≤10 small antral follicles 2-9mm diameter
Derks-Smeets I. A. P., et al. JAssistReprod Genet 2017	***BRCA1* MUTATION CARRIERS HAVE A LOWER NUMBER OF MATURE OOCYTES AFTER OVARIAN STIMULATION FOR IVF/PGD[34]**			**X**	**X**		• **Negative association between *BRCA* status and cryopreservation strategies outcomes** • *BRCA* carriers had lower number of mature oocytes vs control • *BRCA1* carriers had lower number of mature oocytes vs control • No statistically significantly difference in number of mature oocytes in *BRCA2* carriers vs control **Limitations:** sample size (192 women, 18 *BRCA1* carriers vs 20 *BRCA2* carriers vs 154 women with autossomal mutations

							without know association with reduced ovarian reserve); use of different IVF protocols among patients
Gunnala V., et al. Fertility and Sterility 2017	**_BRCA_ MUTATION BREAST CANCER PATIENTS SHOW EQUIVALENT OVARIAN RESERVE AND RESPONSE TO IVF STIMULATION COMPARED TO _BRCA_ NEGATIVE PATIENTS AND OTHER MALIGNANCIES UNDERGOING FERTILITY PRESERVATION.[31]**	**X**	**X**	**X**			• **No association between _BRCA_ status and AMH levels** • **No association between _BRCA_ status and FSH levels** • All cancer cohorts had statistically significantlyly lower FSH levels vs healthy women • **No association between _BRCA_ status and cryopreservation strategies** • *BRCA* carriers had equivalent number of harvested and frozen oocytes vs non-carriers **Limitations:** Demographic disparity among cohorts (3 cancer cohorts younger vs elective cryopreservation group), no account for cancer status as a confounder
Lin Wayne T., et al. Wiley Online Library 2013	**COMPARISON OF AGE AT NATURAL MENOPAUSE IN _BRCA1_/2 MUTATION CARRIERS WITH A NON-CLINIC-BASED SAMPLE OF WOMEN IN NORTHERN CALIFORNIA[42]**				**X**	**X**	• **Negative association between _BRCA_ positive status and parity** • *BRCA* carriers had a higher rate of being nulliparous vs non-carriers • *BRCA* carriers had lower mean parity vs non-carriers • **Negative association between _BRCA_ positive status and menopause onset age** • *BRCA* carriers had lower natural menopause onset age vs non-carriers • *BRCA* carriers had lower (natural or therapy induced) menopause onset age vs non-carriers • No statistically relevant difference in menopause onset age in *BRCA1* carriers vs *BRCA2* carriers

							• **Negative association between smoking and menopause onset age** **Limitations:** sample limited to women from a restricted geographic area (California), potential recall bias
Lambertini M., et al. Annals of Oncology 2018	**REPRODUCTIVE POTENTIAL AND PERFORMANCE OF FERTILITY PRESERVATION STRATEGIES IN *BRCA*-MUTATED BREAST CANCER PATIENTS[25]**	**X**		**X**			• **Negative association between *BRCA* positive status and AMH levels** • *BRCA* carriers had statistically significantlyly lower mean AMH levels vs non-carriers • No statistically significantlyly difference in mean AHM levels in *BRCA1* carriers vs *BRCA2* carriers • **Negative association between *BRCA* positive status and cryopreservation strategies outcomes** • *BRCA* carriers had statistically significantlyly higher percentage of poor response rate to oocyte cryopreservation vs non-carriers • No statistically significantlyly difference in response rate to oocyte cryopreservation between *BRCA1* carriers vs *BRCA2* carriers • *BRCA* carriers tended to have lower number of oocytes per fragment in ovarian tissue cryopreservation vs non-carriers **Limitations:** sample size (156 women, 39 *BRCA* carriers vs 72 non-carriers); small sample of patients who underwent cryopreservation (29 for oocyte and 72 for ovarian tissue)
Oktay K., et al. Journal Of Clinical	**ASSOCIATION OF *BRCA1* MUTATIONS WITH OCCULT PRIMARY**			**X**			• **Negative association between *BRCA1* positive status and cryopreservation strategies outcomes** • *BRCA1* carriers had

Oncology 2010	**OVARIAN INSUFFICIENCY: A POSSIBLE EXPLANATION FOR THE LINK BETWEEN INFERTILITY AND BREAST/OVARIAN CANCER RISKS[36]**						statistically significantlyly higher percentage of low ovarian response rate vs non-carriers • *BRCA1* carriers had 28,7x odds of low ovarian response rate vs non-carriers • *BRCA1* carriers had statistically significantlyly higher percentage of low ovarian response rate vs untested women • *BRCA1* carriers had 38,3x odds of low ovarian response rate vs combined group of non-carriers and untested women • All *BRCA1* carriers with low ovarian response rate were ≥33y.o. • Carriers of only *BRCA2* deleterious mutation presented no low ovarian response • *BRCA* carriers had lower mean of oocytes retrieved vs combined group of non-carriers and untested women **Limitations:** sample size (82 women, 9 *BRCA1* carriers vs 4 *BRCA2* carriers vs 1 *BRCA1* and *BRCA2* carrier vs 33 non-carriers vs 35 untested women); large percentage of the sample had Ashkenazi Jewish ancestry
Gunnala V., et all Fertility and Sterility 2019	**BRCA CARRIERS HAVE SIMILAR REPRODUCTIVE POTENTIAL AT BASELINE TO NONCARRIERS: COMPARISONS IN CANCER AND CANCER-FREE**	**X**	**X**	**X**			• **No association between *BRCA* status in breast cancer patients and AMH levels, FSH levels and cryopreservation strategies outcomes** • **Negative association between breast cancer and FSH levels** • BRCA+ breast cancer patients had lower FSH levels vs non-breast-cancer malignancies patients • BRCA- breast cancer patients

	COHORTS UNDERGOING FERTILITY PRESERVATION[46]						had lower FSH levels vs non-breast-cancer malignancies patients • **Positive association between *BRCA* status and number of mature cryopreserved oocytes** • BRCA carriers had higher number of mature cryopreserved oocytes vs women undergoing elective fertility preservation • BRCA carriers had higher number of mature cryopreserved vs non-carriers • **No association between BRCA status and AMH levels, FSH levels or number of harvested oocytes** • BRCA carriers had no difference in AMH levels, FSH levels or number of harvested oocytes vs women undergoing elective fertility preservation • BRCA carriers had no difference in AMH levels, FSH levels or number of harvested oocytes vs non-carriers • **No association between *BRCA* mutation types and AMH leves, FSH levels total number of harvested oocytes, or number of mature cryopreserved oocytes** • BRCA1 carriers had no differences in any of the markers of ovarian reserve or stimulation response vs BRCA2 carriers **Limitations:** small sample size; cohort disparity (*BRCA* carriers statistically significant younger vs non-carriers; with non-breast-cancer malignancies were statistically significantly younger vs both BRCA+ and BRCA- breast cancer cohorts)
TOTAL		**9**	**3**	**7**	**15**	**4**	

Table 6 – assessment of review articles of interest

AUTHOR	TITLE	OUTCOMES MEASURED					MAIN CONCLUSIONS
		OVARIAN RESERVE			GRAVIDITY AND PARITY	MENOPAUSE ONSET AGE	
		AMH LEVELS	FSH LEVELS	CRYOPRESERVATION STRATEGIES OUTCOMES			
Grynberg M., et al. Future Oncology 2018	**FERTILITY PRESERVATION IN *BRCA*-MUTATED WOMEN: WHEN AND HOW?**[32]	**X**		**X**			• **Possible negative association between *BRCA* positive status and female fertility** • There is conflicting clinical data regarding ovarian reserve assessment through AMH levels and the cryopreservation strategies outcomes in *BRCA* carriers but the negative association constitutes a rational hypothesis upon many research findings
Lambertini M., et al. Cancer Treatment Reviews Cancer Treatment Reviews 2017	**FERTILITY AND PREGNANCY ISSUES IN *BRCA*-MUTATED BREAST CANCER PATIENTS**[56]	**X**		**X**	**X**	**X**	• **Possible negative association between *BRCA* positive status and female fertility** • There is conflicting clinical data regarding ovarian reserve assessment through AMH levels,parity and menopause onset age, with a negative association description mainly on *BRCA1* carriers • There is limited and conflicting clinical data regarding cryopreservation strategies outcomes in *BRCA* carriers, with a possible negative association verified only above *BRCA1* carriers
Smith K. R., et al.	***BRCA1* AND *BRCA2* MUTATIONS AND FEMALE FERTILITY**[44]			**X**	**X**		• **Possible negative or no association between *BRCA* positive status and female fertility**

Current Opinion 2013							• There is conflicting clinical data regarding ovarian reserve assessment through cryopreservation strategies outcomes and gravidity/parity • *BRCA* carriers personal fertility limiting choices is a disregarded confounder in the available studies
Chan J. L., et al. Gynecologic Oncology 2016	**ONCOFERTILITY FOR WOMEN WITH GYNECOLOGIC MALIGNANCIES[58]**			**X**		**X**	• **Possible negative association between *BRCA* positive status and female fertility** • There is conflicting clinical data regarding ovarian reserve assessment and menopause onset age, with a negative association description mainly on *BRCA1* carriers • There is sufficient debate regarding reduced fertility potential, along side management guidelines, to consider the relevance of proposing fertility preservation to *BRCA* carriers
Daum H., et al. Fertility and Sterility 2018	***BRCA* MUTATIONS AND REPRODUCTION[5]**	**X**	**X**	**X**		**X**	• **Possible negative association between *BRCA* positive status and female fertility** • There is conflicting clinical data regarding ovarian reserve assessment and in *BRCA* carriers • There is sufficient debate regarding reduced fertility potential, along side management guidelines, to consider the relevance of proposing fertility preservation
de la Noval B. D., et al.	**POTENCIAL IMPLICATIONS ON FEMALE FERTILITY AND**	**X**		**X**	**X**	**X**	• **Negative association between *BRCA* positive status and female fertility** • Most clinical data regarding ovarian reserve assessment,

Arch Gynecol Obstet 2016	**REPRODUCTIVE LIFESPAN IN *BRCA* GERMLINE MUTATION WOMEN[59]**						parity and menopause onset age show risk of reduced fertility in *BRCA* carriers, with solider evidence on *BRCA1* carriers ≥36y.o. • Early fertility counselling should be included in the management of *BRCA* carriers
Total		**4**	**1**	**6**	**3**	**4**	

Table 7 – assessment of opinion articles of interest

AUTHOR	**TITLE**	**OUTCOMES MEASURED**					**MAIN CONCLUSIONS AND LIMITATIONS**
		OVARIAN RESERVE			**GRAVIDITY AND PARITY**	**MENOPAUSE ONSET AGE**	
		AMH LEVELS	**FSH LEVELS**	**CRYOPRESERVATION STRATEGIES OUTCOMES**			
K. Oktay., et al. J Clin Oncol 2014	**Age-Related Decline in DNA Repair Function Explains Diminished Ovarian Reserve, Earlier Menopause, and Possible Oocyte Vulnerability to Chemotherapy in WomenWith *BRCA* Mutations[60]**	**X**					• **Clinical and basic data support plausibility of negative association between *BRCA* positive status and diminished ovarian reserve, with stronger evidence in *BRCA* carriers >35y.o.**
Peccatori F. A., et al. Human Reproduction 2018	**Fertility preservation in women harboring deleterious *BRCA* mutations: ready for prime time?[61]**	**X**		**X**		**X**	• **There is conflicting clinical data regarding the impact of *BRCA* positive status on ovarian reserve** • **Personalized fertility assessment may be useful during counselling of *BRCA* carriers but it cannot be recommended as part of management strategy for**

							these patients based on current knowledge
Paluch-Shimon, et al. Annals of Oncology 2018	**_BRCA_ 1 AND 2 MUTATION STATUS: THE ELEPHANT IN THE ROOM DURING ONCOFERTILITY COUNSELING FOR YOUNG BREAST CANCER PATIENTS[62]**	**X**		**X**			• **There is reasonable concern and biological plausibility that _BRCA_ carriers may have diminished ovarian reserve and low response to cryopreservation strategies** • **_BRCA_ carriers should be offered fertility preservation**
Total		**3**	**0**	**2**	**0**	**1**	

DISCUSSION

OVARIAN RESERVE MARKERS

A total of 21 articles using ovarian reserve as a predictor of reproductive potential were identified (12 original articles, 6 reviews and 3 opinion articles), resorting to at least one of the following parameters as an outcome: AMH levels, FSH levels and/or cryopreservation strategies.

Considering the non-existence of ideal assessment tools for evaluation of ovarian reserve, the appraisal of the results should bear in mind the inherent limitations.

AMH LEVELS

Among the articles of interest, a total of 9 original articles, 4 reviews and 3 opinions considered ovarian reserve through the evaluation of AMH levels.

The literature described a trend supporting the existence of a negative association between *BRCA* positive status and AMH levels, although there were conflicting results between different workgroups on the implication of the *BRCA* gene mutated. Lambertini M., et al (2018)[25] found a negative association between *BRCA* positive status and AMH levels, with *BRCA* carriers presenting statistically significantlyly lower mean AMH levels compared to non-carriers. Other teams described the existence of a negative association limited to *BRCA1* carriers[26,27], while conflicting data from one group found this association to be only relevant regarding *BRCA2* carriers.[28]

Michaelson-Cohen R., et al (2014)[29] found no difference in mean AMH among *BRCA* carriers when compared with a group of untested women with normal ovulatory cycles and, although described higher levels of AMH in *BRCA1* carriers in comparison to *BRCA2* carriers, this was without statistic relevance. The absence of association between *BRCA* status and AMH levels was reported by other studies.[30,31,46]

The lack of statistical significance was reported by one group, preventing the draw of conclusions.[32]

FSH LEVELS

A total of 3 original articles and 1 review adressed FSH as a means to evaluate ovarian reserve in *BRCA* carriers.

The use of FSH levels as a predictor of low response to IVF is widely regarded as an effective practice, consequently being recommended in multiple guidelines. [17-19] Nonetheless, clinical data suggests this might not be a valid assessment tool in carriers of *BRCA* deleterious variants. Gunnala V., et al. (2017)[31] reported a significant decrease in FSH levels of patients suffering from various cancers when compared to healthy women, however no association between *BRCA* status and FSH was established. More recently, Gunnal V., et al (2019)[46] found no association between BRCA mutational status and FSH levels.

Another workgroup who studied this parameter[33], found that basal FSH levels were similar in both *BRCA* carriers and non-carriers, in spite of not achieving statistical significance and consequently no conclusions were obtained.

CRYOPRESERVATION STRATEGIES OUTCOMES

From the articles of interest, 7 original articles, 6 reviews and 2 opinion articles opted to use the cryopreservation strategies outcomes in order to assess ovarian reserve.

There was inconsistent data in the articles reviewed though a tendency to the establishment of a negative association between *BRCA* positive status and cryopreservation strategies outcomes was noted.

Albeit conflicting results observed regarding some of the parameters evaluated, three studies came to similar results concerning the number of mature oocytes obtained, with *BRCA* carriers presenting lower number when compared to control groups, supporting a negative association between *BRCA* positive status and cryopreservation strategies outcomes.[25,34,35] One finding these groups did not have in common was the difference in response rate depending on the mutated *BRCA* gene. The impact of a specific gene affected was directly evaluated by Oktay K., et al (2010)[36] who reported that carriers of exclusively *BRCA2* deleterious variants did not present low ovarian response and that carriers of *BRCA1* mutations had 28,7x the odds radio of low ovarian response when compared with non-carriers, noting that only *BRCA1* carriers over the age of 33, inclusively, showed low ovarian response. Regarding the improvement of performance of cryopreservation, Turan V., et al (2017)[35] compared protocols with and without the use of Letrozole and outlined that, although the negative association was still present, the combined protocol of Letrozole and rFSH resulted in an improvement of outcome.

The non-existence of association between *BRCA* status and ovarian stimulation performance was reported by three groups[31,32,46], nevertheless this conclusions should take in account possible bias regarding the sample of this studies on account of proven confounders, such as age disparity among groups[31] and exclusion of individuals with characteristics found to affect cryopreservation outcome according to available data.[19,32]

GRAVIDITY AND PARITY

Appraisal of fertility potential through assessment of gravidity and parity was found in a total of 17 articles (14 original articles and 3 reviews).

Review of the results showed intrinsic limitations in most studies by not taking in account confounders such as the intent to conceive and sexuality of the patients, which would have an effect on the natural pregnancy rate registered.

Several studies did not reach statistical significance in order to allow interpretation of the results.[26, 33, 37, 38, 39, 40] The remaining articles presented conflicting data.

The four articles in which a negative association between the presence of a *BRCA* deleterious mutation and gravidity/parity was found reported a higher prevalence of

nulliparity among *BRCA* carriers when compared with non-carriers[27,41,42] and a higher percentage of history of infertility when compared with low-risk controls.[28]

Three analysis found no association between *BRCA* status and parity, although both reported a statistically relevant higher self-report of infertility in *BRCA* carriers vs non-carriers.[30,43]

A positive association between *BRCA* positive status and gravidity/fertility was stated by two research teams.[44,45] These findings, although not disregardable, should be carefully considerate due to limitations regarding the inclusion criteria applied in both studies. The decision to have children may be influenced by the carrier condition, the previous familiar or personal history of cancer.

MENOPAUSE ONSET AGE

A total of 8 articles (4 original articles, 3 reviews and 1 opinion article) assessed *BRCA* carriers' fertility potential based on menopause onset age considering it marks the end of reproductive lifespan.

Considering the current recommendation of bilateral adnexectomy in *BRCA* carriers[8,9,10], the data on natural menopause onset might be difficult to assess.

A negative association between *BRCA* positive status and menopause onset age was observed in two studies, where *BRCA* carriers experienced menopause earlier when compared with non-carriers.[37,42] Finch A., et al (2013)[37] found that the prevalence of women who had undergone menopause before the age of 40 years old was higher in *BRCA* carriers, which may reflect a lower ovarian reserve or accelerated atresia of follicles.

Tilborg T. C., et al (2016)[41] reported no association between *BRCA* status and menopause onset age, with *BRCA* carriers and non-carriers presenting similar risk of early menopause.

As previously stated, smoking constitutes a risk factor for ovarian aging and this negative association was supported by Collins I. M., et al (2013)[38]. Conversely, this group could not find statistical significance regarding the association between *BRCA* status and natural menopause onset age.

CONCLUSIONS

Clinical data regarding the possible association between female reproductive potential and deleterious variants of *BRCA1*/*BRCA2* genes has presented conflicting results. Nonetheless, most of the available information regarding ovarian reserve, parity and menopause onset age supports the existence of a negative association, leading to the consideration of fertility impairment as an additional consequence and outcome of *BRCA* mutations and raising the need of inclusion of fertility preservation strategies in the management of these patients by offering the option of personalized fertility assessment.

Considering the lack of data regarding the Portuguese population about this matter and the presence of *BRCA* deleterious variants specific to this community, studies should be conducted in order to assess the pertinence of the inclusion of such fertility counselling in the national management strategies of *BRCA* carriers.

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